CHAPTER 1

INTRODUCTION

Nanotechnology is the modern technology that has become an important means for manufacturing new class of materials with numerous applications in science and technology. The nanometer size particles exhibit novel physicochemical, magnetic, and optoelectronic properties that are governed by their size, shape and homogeneity. Among the credible number of nanoparticles, metallic nanoparticles are considered as the most promising materials for a number of applications ranging from as catalysts, chemical sensors, electronic components, medical diagnostic imaging and pharmaceutical products to medical treatment protocols [1]. In particular, silver nanoparticles have attracted much attention due to its wide utility in the field of medicine, pharmaceutics, food packaging, water treatment and wound dressings. Although silver nanoparticles have elegant features, its production methods is an important issue. In order to stabilize and control the nanoparticle morphologies various polymers [2], biological macromolecules [3], latex particles [4], mesoporous inorganic materials [5], dendrimers [6], microgels or hydrogels [7], colloidal systems [8], and others were used. In recent years, the concept of green chemistry and ecofriendly biological processes for nanoparticles synthesis was developed in which biological entities behave not only as reducing agent but also stabilizes the
formed nanoparticles. Among the recognized novel approaches, hydrogels have been used as promising templates or nanopots to prepare nanoparticles that brought a concept for newer composite / hybrid materials [9]. Moreover, these nanocomposite systems possess, by synergetic effects between their organic and inorganic components, new properties such as enhanced mechanical toughness, large deformability, high swelling / deswelling rates, excellent electrical conductivity, high transparency and remarkably strong antibacterial activity in combination with a fairly low toxicity against human tissues.

1.1. HYDROGELS

A gel is a colloidal system constituting the solid as the dispersion medium and the liquid or gas as the dispersed phase. The solid dispersion medium form, by the interlocking of the particles, a loose three dimensional framework that spans large volume of a dispersant and entangles it through surface tension effects. If the dispersant is a gas then the system is called aerogel and lyogel if it is a liquid.

Gels which are physically, non-covalently crosslinked networks that are stabilized by Van der Waal’s interaction, hydrogen bonding or charge attraction are known as physical gels. These gels are stable but can easily be reliquified. In contrast, chemical gels are covalently crosslinked and are stabilized by bonds. They do not reliquify but looses the accumulated fluid,
turning back into a solid. Virtually this process is reversible for any fluid and if this happens with water then the gel is called hydrogel.

Hydrogels are the most distinctive class of three dimensional polymeric networks which possess good hydrophilic character and can hold large quantity of aqueous solutions without undergoing dissolution. In hydrogels, the minimum amount of water present is about 10% of its total weight and when the quantity exceeds 95% of its total weight, then the hydrogel is called a superabsorbent. The hydrophilicity of the hydrogel is due to the presence of functional groups such as –OH, –COOH, –NH₂, –CONH₂, –SO₃H within the polymer backbone or in lateral chains and its stability and insolubility are due to three dimensional networks developed by either physical or chemical cross-linking. These networks not only prevent the hydrogels from dissolution but also make them to swell in aqueous solutions [10-12]. Completely swollen hydrogels portrays the balance between dispersing and cohesive forces in that gel. Various interactions such as electrostatic, dipole – dipole and covalent cross-linking are responsible for the generation of cohesive forces [13-14]. The hydrogels possess numerous advantages over inorganic materials such as lower material cost and easy fabrication, better biocompatibility and biodegradability and more versatile processability and functionalization [15]. Their soft, flexible nature, alikeness to natural living tissue,
hypotoxicity [16] and inherent biocompatibility have made hydrogels suitable for pharmaceutical applications such as oral drug delivery [17], wound dressing [18-19], tissue engineering [20] and so on.

These hydrogels respond to various external stimuli such as pH [21], temperature [22], pressure [23], electric field [24] and undergo structural change by deformation, swelling, dissociation of functional groups etc. The stimuli responsiveness of these hydrogels have been exploited in the preparation of “smart or intelligent gels” which, owing to certain self-controlled mechanisms, find extensive applications in the fields of biomaterials, sensors, biosensors[25], medicines [26], separation and enrichment technologies [27] and chemical valves. Structurally, smart hydrogels show resemblance to the extracellular matrix of various tissues and can often be processed under moderately mild conditions and may be delivered in a minimally invasive manner. These properties can be advantageously utilized to make scaffold materials for drug delivery [28], engineering tissue replacement and other applications [29].

The incorporation of inorganic systems, particularly nanometals, in the three dimensional network of hydrogels enhances certain properties significantly. This results in the intensive investigation of new hybrid materials known as composites. Since silver is an antibacterial agent showing strong cytotoxicity for various microorganisms and has been
extensively used to control infections since ancient times [30-32], it was frequently selected for preparing nanocomposite materials. Although nanosilver can be made using various physicochemical methods [33-34], their synthesis using nontoxic and environmentally friendly biological methods is attractive especially if they are intended for invasive applications in medicine. An ideal hydrogel has the following functional features

- Maximum equilibrium swelling in saline media
- Desired rate of absorption (chosen particle size and porosity) based on the requirement
- Maximum absorbency under load (AUL)
- Minimum solubility and residual monomer
- Cheaper in price
- Highest durability and stability in the swelling atmosphere and during storage.
- Utmost biodegradability without the formation of toxic species on degradation
- pH neutrality after swelling in water
- Colorlessness, odorlessness, and absolute non-toxic
- Stability to light
- Re-wetting capability i.e., the ability to give back the swollen liquid or to maintain it.
1.1.2. PROPERTIES OF HYDROGELS

Hydrogels are generally synthesised from natural and synthetic polymers and have unique properties that have been improvised by functionalization and reinforcing to produce its variety of forms as modified hydrogels, composites and nanocomposites. These materials possess characteristic properties like swelling behaviour, mechanical properties and biocompatibility.

1.1.2.1. Swelling characteristics

Hydrogels have polymeric chains that are cross-linked together either physically (entanglements, crystallites) or chemically (tie-points, junctions) and behave as one molecule regardless of its size. Hence, they are sometimes called as infinitely large molecules or super macromolecules. The tendency of hydrogels to expand in water is because of the hydrophilic groups present in the polymeric chains and its mechanical resistance is due in part to the physical or chemical cross-linking. Any change in external parameters may lead to a change in physical texture of the hydrogel. These changes may occur because of either fall in the number of mobile ions inside the networks which cause decrease in osmotic pressure and hence the swelling capacity or due to anion–anion repulsive electrostatic forces.
1.1.2.2. Mechanical properties

Mechanical properties of hydrogels are very important for designing a therapeutic system. For biomedical applications viz. wound dressing material, matrix for drug delivery, ligament and tendon repair and tissue engineering, it is essential that the carrier gel matrix maintain its physical integrity and mechanical strength for the predetermined period of time. The strength of the gel can be increased by increasing the degree of cross-linking and introduction of co-monomers. However, this decreases the elasticity of the hydrogel and makes it more brittle. Elasticity is required to give flexibility to the cross-linked chains of the hydrogel in order to facilitate the movement of incorporated biomaterials. Hence, it is necessary to maintain an optimum degree of cross-linking to achieve a relatively strong and yet elastic gel.

1.1.2.3. Biocompatible properties

Biocompatibility is the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient of that therapy, but generating the suitable beneficial cellular response in that specific situation and optimizing the clinically relevant performance of that therapy [35]. Hydrogels have to be biocompatible and nontoxic in order to be applied in biomedical field. For the same reason, they must pass the cytotoxicity and in-vivo toxicity
tests. Thus, an evaluation of the inherent toxicity of all materials used for the synthesis of a gel is an integral part of determining appropriateness of the gel for biological applications. Toxic chemicals used during the synthesis of a gel affect biocompatibility if left unreacted at the end of the reaction. Furthermore, cross-linkers, redox initiators, polar solvents, stabilizers, emulsifiers and unreacted monomers may be toxic to host cells if they leach out to tissues. To decrease the chances of toxic effects, special techniques and various purification processes are used. Also, kinetics of polymerization has been studied in order to achieve higher conversion rates to avoid unreacted monomers and side products.

1.1.3. Classification of hydrogels

Hydrogels can be classified on different bases (Fig. 1.1.) and some of them are discussed below.

1.1.3.1. Classification based on source

Hydrogels can be classified into three categories based upon their origin viz., natural, synthetic and semi-synthetic.

**Hydrogels based on natural polymers**

Natural hydrogels are based on natural polymers such as polypeptides, polysaccharides, polynucleotides etc. These natural polymers are obtained from various natural sources. For example, collagen is derived
Figure 1.1. Classification of hydrogels

from mammals whereas almond gum exudates from the trunk of the almond tree. Advantages of these natural polymers include biodegradability, biocompatibility, non-toxicity and easily modified ability than various synthetic polymers. Although natural polymers have many advantages over synthetic polymers, still their mechanical and degradation properties are less controlled and they also provoke a serious immune response.
Hydrogels based on synthetic polymers

Synthetic hydrogels are based on synthetic polymers like polyvinyl alcohol, polyacrylic acid, polyhydroxyethyl methacrylate etc. They can be modified to have a much wider range of mechanical and chemical properties than their natural complements. Most of the hydrogels are biocompatible and are synthesized by traditional polymerization of vinyl-activated monomers.

Hydrogels based on semi-synthetic polymers

Modified hydrogels based on both natural and synthetic polymers are synthesised in order to combine the advantages of both synthetic and natural polymers. In these hydrogels, basic chemical structure is modified by copolymerization, grafting or blending so that additional functionalities can be added to provide tailor made properties in the hydrogel for any particular application. Temperature-responsive hybrid hydrogels have been prepared by combining natural polymers with poly(N-isopropyl acrylamide). pH-sensitive hybrid hydrogels have been prepared by combining natural polymers with poly (acrylic acid).

1.1.3.2. Classification based on polymeric composition

Hydrogels can be classified into homopolymeric, heteropolymeric and copolymeric, based on the method of preparation.
Homopolymeric hydrogels

Homopolymeric hydrogels are cross-linked networks resulting from a single species of monomer unit [36]. Depending on the nature of the monomer unit and adopted polymerization method, they may have cross-linked skeletal structure. These hydrogels are applied in drug delivery systems, wound dressings, artificial skin manufacturing and contact lenses.

Heteropolymeric hydrogels

In heteropolymeric hydrogels, cross-linked polymer networks are derived from two or more polymeric chains (natural, synthetic or both) and are further classified into

➢ Interpenetrating polymer networks (IPN)

➢ Semi interpenetrating polymer networks (Semi-IPN)

Interpenetrating polymer networks

IPN is the polymer comprising of two or more networks which are partially interlocked on a molecular scale but not covalently bonded to each other and cannot be separated unless chemical bonds are broken”. A mixture of two or more pre-formed polymer networks is not an IPN [37].

Semi interpenetrating polymer networks

Semi IPN is the polymer comprising of one or more networks and one or more linear or branched polymer(s) characterized by the infiltration on a
molecular scale of at least one of the networks by a minimum number of linear or branched macromolecules”.

**Copolymeric hydrogels**

In copolymeric hydrogels, two polymer chains are connected covalently as blocks, in a random fashion, in alternate order and as grafts [38]. The properties of the hydrogels network depend on the varied combination of polymeric units and particular arrangements of these polymeric chains.

1.1.3.3. **Classification based on spatial arrangement**

Hydrogels are classified based on the spatial arrangement of the network as amorphous, semicrystalline, crystalline and hydrocolloid aggregates.

**Amorphous**

In amorphous hydrogels, the macromolecular chains are arranged randomly. Examples for amorphous hydrogels include hydrophilic polymer paste used for wound dressing and as a carrier for many topical drugs like metronidazole.

**Semicrystalline**

Semicrystalline hydrogels are the complex mixture of amorphous and crystalline phases and are characterized by dense regions of ordering macromolecular chains. The properties of semicrystalline hydrogels are
studied by degree of crystallinity and size as well as orientation of molecular chains.

**Crystalline**

Crystalline hydrogels are not free-flowing gels and have compact structure. HYPAN, for example, has physical network of crystalline clusters and are used in controlled drug delivery.

**Hydrocolloid aggregates**

Hydrocolloid gels consist of dispersed phase (polysaccharide or protein) and dispersing medium (water). They show very close resemblance to a liquid or to a solid. The liquid like character arises due to high percentage of water and the solid like property is due to the formation of network and characterized by a finite elastic modulus.

1.1.3.4. **Classification based on nature of cross-linking**

Hydrogels can be divided into physical hydrogel and chemical hydrogel based on the physical or chemical nature of the cross-linked networks [39].

**Physical hydrogel**

Physical gels have transient junctions and are formed either by molecular entangled chains or physical interactions such as hydrogen bonds, hydrophobic interactions and ionic interactions.
Chemical hydrogel

Chemical gels have permanent junctions and are formed by covalent bonds. They are commonly water swollen networks of hydrophilic homopolymers or copolymers.

1.1.3.5. Classification based on physical appearance

Hydrogels can be categorised as matrix, film or microsphere based on the method of polymerization followed in the preparation process.

Hydrogel matrix

Hydrogel forms a matrix when a network of cross-linked polymeric chains create a three-dimensional structure with interstitial space that are filled up with water and often biological fluids.

Hydrogel film

A simple solution-casting technique was used to prepare hydrogel films with various thickness at room temperature.

Hydrogel microsphere

A novel precipitation polymerization technique was used to prepare small and monodisperse hydrogel microspheres.

1.1.3.6. Classification based on network electrical charge

Hydrogels can be categorized as neutral, ionic, ampholytic and zwitter ionic on the basis of presence or absence of electrical charge located on the crosslinked chains.
1.1.3.7. Classification based on biodegradability

Hydrogels can be classified into biodegradable and non-biodegradable based on their solubility in biological fluids and water.

**Biodegradable**

Biodegradable hydrogels have the capability of breaking down to simpler molecules, inside the body, with both water and enzymes.

**Non-Biodegradable**

Non-biodegradable hydrogels are not broken down by the water and enzymes [40].

1.1.3.8. Classification based on the presence or absence of water

Based on the presence or absence of water, they can be categorized as organogel, xerogel, aerogel, oleogel and bigel.

**Organogel**

Organogel comprises semi-solid systems, in which an organic liquid phase is immobilized by a three-dimensional network poised of self-assembled, intertwined gelator fibres. It is a type of hydrogel that has non-crystalline, thermoplastic solid material which is composed of a liquid organic phase entangled in a three dimensional cross-linked network [41].

**Xerogel**

Xerogel is a dry form of a hydrogel. It is a solid that is formed from a hydrogel by drying it with unobstructed shrinkage. They are highly porous...
(15 to 50%) in nature along with tiny pore sizes (1–10 nm). They have enormous surface area (150 to 900 m$^2$/g) [42].

**Aerogel**

Aerogel is derived from a gel by the technique of supercritical drying. Here the liquid part of the gel is replaced with a gas. It is effective as a thermal insulator and has extremely low density [42].

**Oleogel**

Oleogels are lipophilic liquid and solid mixtures, in which solid lipid materials (oleogelators) with lower concentrations (<10 wt. %) entrap bulk liquid oil by the ways of the formation of network of oleogelators in the bulk oil [43].

**Bigel**

Bigels are the amalgamation of oleogel and hydrogel mixtures [44].

1.1.3.9. **Classification according to their physical properties**

Hydrogels can be classified into two groups on basis of their responses according to the changes in environment.

**Conventional hydrogels**

Conventional hydrogels are made by the cross-linking of the polymers and they do not show any response according to the change in environment.
Smart hydrogels

Smart hydrogels are also known as stimulus-responsive polymeric hydrogels. Smart hydrogel is defined as the polymer network able to respond to external stimuli through abrupt changes in the physical nature of the network. They respond with sharp and large property changes in response to small change in physical or chemical conditions. Types of stimuli include pH, temperature, magnetic field, sound, glucose, ionic strength, solvent composition, pressure, electrical potential, radiation, chemical and biological agents [45].

1.1.3.10. Classification based on porosity

An important feature of hydrogel swelling is the rate of swelling which is ascertained by various physicochemical parameters particularly the extent of porosity and the kind of porous structure. On this basis, hydrogels can be classified as non-porous, micro-porous, macro-porous and super-porous hydrogels.

1.1.4. Review of literature

It is known that hydrogel networks can function as effective carriers for nanosystems and natural polymers (polysaccharides, mineral clays etc.) as renewable prepolymer materials. Thus, this part of the thesis focuses on the recent development in various synthetic strategies used for the production of hydrogels, silver nanoparticles and hydrogel silver
nanocomposites. A brief account of the wide application of these materials in agriculture, pharmaceutical and biological systems along with the literature survey related to the present study is also included.

1.1.5. Preparation methods of hydrogels

Hydrogels are the polymeric materials possessing excellent hydrophilic character. They can swell and imbibe large amount of water without dissolution. The ability of hydrogels to absorb water is due to the presence of hydrophilic groups that are attached to the polymeric backbone while their resistance to dissolution is attributed to cross-linking that exists within the polymeric networks. They are predominantly used in biomedical and tissue engineering applications. In such applications, the three dimensional matrix have to disintegrate into non-dangerous products to ensure biocompatibility of the hydrogel. The nature of the degradation products can be tailored by the proper selection of the hydrogel building blocks. With this knowledge, polymers of both natural and synthetic origin are cross-linked to form novel hydrogels adopting several methods.

1.1.5.1. Cross-linking of polymers

Cross-linking of polymeric chains can be done by

i. chemical reactions

ii. high energy radiation

iii. enzymes
iv. ionic interactions
v. crystallization
vi. hydrogen bonds
vii. protein interaction

Among the techniques mentioned above, the top three produces chemically cross-linked hydrogels and the remaining four gives physically cross-linked hydrogels.

1.1.6. Applications of hydrogels

Hydrogels have attracted much attention due to their high water absorption capacity and biocompatibility. In addition to the swelling characteristics, the soft, flexible nature, alikeness to natural living tissue, hypotoxicity and biodegradability have made hydrogels suitable for the development of a variety of smart devices for specific applications. The following list gives some of the potential fields in which hydrogel technologies can be applied

- Personal hygiene products
- Agriculture
- Drug delivery systems
- Sealants
- Coal dewatering
- Artificial snow
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- Food additives
- Pharmaceuticals
- Biomedical applications
- Tissue engineering and regenerative medicines
- Diagnostics
- Wound dressing
- Separation of biomolecules or cells
- Barrier materials to regulate biological adhesions
- Biosensor
- Implants
- Injectable polymeric systems
- Hybrid type organs
- Dental materials
- Ophthalmic applications
- Trans-dermal systems

1.2. Silver nanoparticles

As mentioned earlier, nanotechnology has become vital tool for the invention of newfangled materials with numerous applications in science and technology. These materials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment. Metal nanomaterials including nano clusters,
nano wires and related nanostructures have received tremendous attention owing to their unique catalytic, electrical, magnetic and thermal properties. Among them, nano-sized silver and its derivatives have attracted many researchers as an antibacterial and antifungal agent over several decades. Both silver ions and silver nanoparticles could disrupt the structure of the cell membrane of the microbial species and cause inhibition of their growth. Moreover, the minimum inhibitory concentration of nano-sized silver was lower than the cytotoxicity level of tested human cells.

1.2.1. Synthesis of silver nanoparticles

Numerous synthesis methods were developed to obtain silver nanoparticles of desired size and shape [46]. Each method has advantages and disadvantages and may be broadly classified into

- Physical methods
- Chemical methods
- Bio methods

1.2.1.1. Physical methods

Evaporation condensation method

Silver nanoparticles are usually prepared by evaporation condensation, which could be accomplished using a tube furnace at atmospheric pressure. A boat centered at the furnace is used to vaporize the source material into a carrier gas. The carrier gas is then cooled at a suitable
rate. Utilizing this method, small AgNPs in high concentration can be achieved but it has several drawbacks like consumption of great deal of energy and time [47-50].

**Laser ablation method**

AgNPs have been synthesised with laser ablation of metallic bulk materials in solution [51-52]. An advantage of this method compared to other conventional methods for preparing NPs is the absence of harmful chemical reagents in solutions. In this method, pure NPs, which will be useful for further applications, can be prepared.

**Arc discharge method**

Electrical arc discharge of electrodes submerged in deionized water is an attractive technique to synthesize AgNPs because of simplicity of experimental setup, purity of the product and cost-effective procedure. In this method, direct current is applied between two silver rods which are immersed in ultrapure water at room temperature without any heat exchanger, vacuum and gas handling equipment.

Tien et al. [53] synthesised silver nanoparticles by arc discharge in aqueous solutions. In their study, two silver electrodes were melted by high temperature caused by joule heating and converted into silver nanoparticles. Lo et al. [54] reported the use of ethylene glycol instead of pure water to achieve a narrow particle size distribution.
1.2.1.2. Chemical methods

Reduction method

It is the most common method for the synthesis of AgNPs because of its convenience, simple experimental setup, control over the growth of NPs, low cost and high yield. In general, the synthesis process of AgNPs in solution usually involve the following three main elements:

i. metal precursors

ii. reducing agents

iii. capping agents

The formation of colloidal silver from the reduction of silver salts involve two stages viz. nucleation and subsequent growth [55]. The size and shape of the synthesised AgNPs are strongly dependent on these stages.

The common reducing agents used for the synthesis of AgNPs includes sodium citrate, sodium borohydride, hydrogen, Tollen's reagent, N,N-dimethyl formamide and poly (ethylene glycol)-block copolymers. These agents converts Ag$^+$ ions into Ag$^0$, which is followed by the aggregation of metallic silver into oligomeric hunks. These hunks eventually lead to the formation of colloidal silver particles [56-58].

D. Radziuk et al. [59] reported the synthesis of AgNPs by reduction of silver nitrate solution in an excess of aqueous sodium borohydride. The nanoparticles with a diameter of 20 nm are obtained. Luo et al. [60]
prepared AgNPs under mild conditions using polyethylene glycol. During the reduction process, the reducing rate of Ag\(^+\) ions into nano-Ag was increased remarkably with the increase of polymer chain length of PEG.

S.L.C. Hsu and co-workers [61] used formaldehyde as reducing agent, polyvinyl pyrrolidone (PVP) as stabilizer and organic bases as the reaction promoter to produce AgNPs suspensions. Nearly monodisperse AgNPs were prepared using a simple oleylamine-liquid paraffin system by Chen and colleagues [62]

**Microemulsion technology**

The microemulsions are very good reaction media for the synthesis of AgNPs because the steric barrier by the surfactant monolayer restricts the growth of precipitated particles and hinders intergrain aggregation. The NPs are generally synthesised by mixing one microemulsion containing Ag ions with another microemulsion that contains the reducing agent. Collisions and coalescence of droplets result in the reduction of Ag ions inside the water core and gives a Ag precipitate. Zhang et al. [63] prepared colloidal AgNPs via the reduction of silver nitrate by hydrazine hydrate in the water cores of two separate microemulsions made of dodecane and AOT.

**Photoreduction process**

The photo-induced synthetic strategies have been developed for the synthesis of AgNPs. For example, Huang and Yang prepared AgNPs
through photoreduction of silver nitrate in layered inorganic laponite clay suspensions [64]. On irradiation of photons, disintegration of AgNPs into smaller size with narrow distribution were achieved.

**UV-initiated photoreduction**

Photoreduction using UV radiation has been reported for the synthesis of AgNPs in the presence of citrate, polyvinyl pyrrolidone, poly(acrylic acid) and collagen. Yang et al. [65] reported the synthesis of spherically shaped AgNPs based on the reduction of silver nitrate by a UV-irradiated arginine-tungstosilicate acid solution. Huang et al.[66] synthesised AgNPs in an alkaline solution of $\text{AgNO}_3 / \text{carboxymethylated chitosan}$ utilizing UV radiation.

**Electrochemical method**

Electrochemical method can be applied to synthesize size controlled AgNPs by adjusting electrolysis parameters. It is also possible to increase the homogeneity of AgNPs by changing the composition of electrolytic solutions. Khaydarov et al. [67] synthesized AgNPs of 10 nm size using an inexpensive two-electrode setup in which both anode and cathode are silver wires placed vertically, face-to-face, 10 mm apart. The electrodes are submerged into an electrochemical cell filled with deionized water and electrolysis was performed for 1 h at the temperature range of 325 - 340 K with a constant voltage of 20 V.
Microwave-assisted synthesis

For the synthesis of AgNPs, microwave heating has been reported recently to have better promising method over conventional thermal heating. The advantages of microwave assisted synthesis include shorter reaction times, less energy consumption and higher yields. In the microwave energy range, polar molecules try to align with the electric field. When the dipolar molecules try to re-align with respect to an alternating electric field, they lose energy in the form of heat [66].

Wang et al. [68] described the formation of AgNPs by microwave irradiation of silver nitrate solution with carboxymethylchitosan as reducing as well as stabilizing agent. Vongehr et al. [69] analyzed current progress in the exploitation of various water-based synthesis routes towards the shape-controlled synthesis of AgNPs and microstructures. Numerous one-pot methods utilizing commercial microwave ovens were described.

1.2.1.3. Biosynthetic methods

Nowadays, biological methods using naturally reducing agents such as biodegradable polymers, polysaccharides, microorganisms or plants extract have evolved as a simple and efficient alternative to more complex chemical and physical methods to obtain AgNPs. Moreover, synthesis of nanoparticles by chemical methods have potential environmental hazards. Thus, there is a increasing demand to develop eco-friendly processes, which
restricts the use of toxic chemicals during the synthesis. Greener synthesis of nanoparticles also provide advantages over other methods as they are simple, one step, cost-effective, eco-friendly, relatively reproducible and often results in more stable products.

**Biodegradable polymers**

Polymers that possess ion-exchangeable capability can be used in synthesizing AgNPs. Commonly used polymers contain phosphonic acid groups and have low molecular weight. Initially, a complex was formed between the polymer and Ag\(^+\) ions and then the Ag\(^+\) ions were reduced into silver nanoparticles. The formed AgNPs were also stabilized by the ion-exchange polymer. The surface anatomy showed the presence of cubes and rectangular prisms [70]. Copolymers like cyclodextrin grafted poly (acrylic acid), can be used to produce nanosized silver where potassium persulfate was used as an initiator. The copolymer reduces and stabilizes the silver ions that results in the formation of AgNPs, thus, these nanoparticles aggregated. The concentration of the base, AgNO\(_3\), the copolymer and the method of heating played a role in determining the size of the congregated nanoparticles [71].

**Polysaccharides**

Among the substances used in various techniques for the synthesis of AgNPs, the polysaccharides have been shown to be an excellent candidates
for stabilizing and controlling the size of nanoparticles. The stabilization provided by polysaccharides rely on the presence of multiple binding sites along the polysaccharide chain to facilitate attachment to the metal’s surface, thereby effectively “trapping” the metal nanoparticle and conferring significant protection against aggregation and chemical modification.

Vigneshwaran et al. [72] reported the synthesis of stable silver nanoparticles by the use of polysaccharides, such as starch. Hydrogen bonding present in starch protects the agglomeration of formed AgNPs. Glucose, silver nitrate, starch and sodium hydroxide are used to act as reducing agent, precursor, stabilizer and accelerator respectively, for the reduction synthesis of silver nanoparticles. Tran et al. [73] has obtained monodisperse AgNPs with highly antibacterial and antiproliferative activities by a simple but green synthesis using non-toxic chitosan as both reducing and stabilizing agents. In the process of Ag⁺ reduction, chitosan played a role of reductant and the reduction of Ag⁺ was coupled to the oxidation of the hydroxyl groups in molecular chitosan and/or its hydrolyzates. The polymer, chitosan, is natural and is a good agent for chelating and stabilizing, thus making this an eco-friendly approach.

Tollens reagent is known to act as reducing agent for the synthesis of size controllable AgNPs. This environmental friendly synthesis involves reduction of Ag(NH₃)⁺² by an aldehyde. In the modified Tollens method,
Ag(NH$_3$)$_2$$^{+2}$ ions are reduced using the saccharides like glucose, galactose, maltose and lactose [74] to obtain AgNPs measured between 50 and 200 nm.

**Bacteria**

Bacteria are the potential biofactories for the production of silver and gold nanoparticles. Synthesis of NPs by microorganisms is due to their defence mechanism. The resistance caused by the bacterial cell for silver ions in the environment is responsible for its NPs synthesis. It is possible to synthesize highly stable AgNPs by the bioreduction of aqueous silver ions with a culture supernatant of non-pathogenic bacterium, *Bacillus licheniformis* [75].

Samadi et al.[76] developed an efficient and eco-friendly viable process for the synthesis of nanoscale silver particles using *Proteus mirabilis* PTCC 1710, a bacterial strain that was isolated during a screening program from photographic waste. A significant outcome of this study is their observation that the silver nanoparticles could be induced to synthesis intra and extracellularly.

**Fungi**

AgNPs can be prepared through a novel biological approach using the fungus *Verticillium*. The Ag$^+$ ions, on exposure to the fungal biomass undergoes reduction to give silver nanoparticles with dimensions of
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~ 25 nm. Most likely the silver ions were reduced by the enzymes present in the membrane of the cell wall. An important advantage of this method is that the fungal cells still continued to grow even after the biosynthesis of the silver nanoparticles, which shows that they are non-toxic [77]. Another fungal species studied was *Trichoderma asperellum*, which is the common culturable fungi found in soil. It is the non pathogenic and agriculturally important fungus which can be used to synthesize highly stabilized nanocrystalline silver particles ranging between 13 to 18 nm. The silver nanoparticles stay at a constant shape and size even after six months of being stored [78].

Kathiresan and colleagues [79] have revealed that when the culture filtrate of *Penicillium fellutanum* was incubated with silver ions and maintained under dark conditions resulted in the formation of spherically shaped AgNPs.

Algae

El-Rafie et al. [80] demonstrated a novel environmentally safe method for the synthesis of AgNPs using water soluble polysaccharides extracted from four marine macro-algae, namely, *Pterocladia capillacea*, *Jania rubins*, *Ulva faciata*, and *Colpomenia sinusa*. These polysaccharides have a dual effect as they act as reducing agents for silver and as stabilizing agents for the formed AgNPs.
D.A.d. Silva and co-workers [81] reported the preparation of AgNPs using a naturally occurring algal polysaccharide isolated from red marine algae (Gracilaria birdiae), which acted as both the reducing and stabilizing agent. The hydrodynamic width of the AgNPs varied from 20.2 nm to 94.9 nm.

**Plants extract**

Extracts of various plants that are environmental friendly and economically cheap materials have been explored in the preparation of AgNPs. All parts of a plant bearing antioxidants or sugars, including leaves, fruits, roots, seeds, and stems can be used in the synthesis process. A large number of plants are reported to facilitate silver nanoparticles syntheses and are mentioned in Table 1.1 [82].

**Table 1.1. Synthesis of silver nanoparticles using plant extracts.**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Size (nm)</th>
<th>Plant’s part</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternanthera dentate</td>
<td>50–100</td>
<td>Leaves</td>
<td>Spherical</td>
</tr>
<tr>
<td>Acorus calamus</td>
<td>31.83</td>
<td>Rhizome</td>
<td>Spherical</td>
</tr>
<tr>
<td>Boerhaavia diffusa</td>
<td>25</td>
<td>Whole plant</td>
<td>Spherical</td>
</tr>
<tr>
<td>Tea extract</td>
<td>20–90</td>
<td>Leaves</td>
<td>Spherical</td>
</tr>
<tr>
<td>Tribulus terrestris</td>
<td>16–28</td>
<td>Fruit</td>
<td>Spherical</td>
</tr>
<tr>
<td>Cocous nucifera</td>
<td>22</td>
<td>Inflorescence</td>
<td>Spherical</td>
</tr>
<tr>
<td>Abutilon indicum</td>
<td>7–17</td>
<td>Leaves</td>
<td>Spherical</td>
</tr>
<tr>
<td>Pistacia atlantica</td>
<td>10–50</td>
<td>Seeds</td>
<td>Spherical</td>
</tr>
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<td>8–40</td>
<td>Leaves</td>
<td>Spherical</td>
</tr>
<tr>
<td>Ficus carica</td>
<td>13</td>
<td>Leaves</td>
<td>–</td>
</tr>
<tr>
<td>Cymbopogan citratus</td>
<td>32</td>
<td>Leaves</td>
<td>–</td>
</tr>
<tr>
<td>Acalypha indica</td>
<td>0.5</td>
<td>Leaves</td>
<td>–</td>
</tr>
<tr>
<td>Premna herbacea</td>
<td>10–30</td>
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<td>Spherical</td>
</tr>
<tr>
<td>Calotropis procera</td>
<td>19–45</td>
<td>Plant</td>
<td>Spherical</td>
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<tr>
<td>Centella asiatica</td>
<td>30–50</td>
<td>Leaves</td>
<td>Spherical</td>
</tr>
<tr>
<td>Argyreia nervosa</td>
<td>20–50</td>
<td>Seeds</td>
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1.2.2. Applications of silver nanoparticles

Silver nanoparticles are one of the most used materials in the ever expanding nanotechnology sector. The extensive use of AgNPs is because of the unique and altered physical, chemical and biological properties at the nano scale due to their high surface-to-volume ratio. Thus, these nanoparticles have been the topic of considerable research in recent years.
Introduction

[83-86]. AgNPs have been used widely as anti-bacterial agents in the health engineering, food storage, textile coatings and a number of environmental applications. It is noteworthy that in spite of AgNPs long term use, the evidence of toxicity of silver is still not apparent. An overall view of the applications of silver nanoparticles is given in Fig 1.2.

Figure 1.2. Applications of silver nanoparticles

1.3. Hydrogel silver nanocomposites

The synthesis of nanocomposites by combining metal nanoparticles with soft, expandable three dimensional hydrogel networks have gained significant attention, owing to their fascinating optical, electrical and catalytic properties. The introduction of nanomaterials into the hydrogel matrix endow with new properties, which are not available to the conventional hydrogels. So far, many nanomaterials have been used in the
development of hydrogel nanocomposites including metallic nanoparticles, carbon nano tubes, clay materials, magnetic nanoparticles, hydroxyapatite and semiconducting nanoparticles. Therefore, the hydrogel nanocomposites are classified based on the type of nano particulate systems used.

1.3.1. Classification of hydrogel silver nanocomposites

1.3.1.1. Clay nanocomposites

One of the most widely explored classes of hydrogel nanocomposites involve the addition of clay nanoparticles into the hydrogel system. Liang et al. [87] have shown faster swelling transition and increased release rate with a poly(N-isopropylacrylamide)/clay nanocomposite hydrogel through successful dispersion of the clay throughout the nanocomposite.

1.3.1.2. Hydroxyapatite nanocomposites

Hydroxyapatite nanocomposites are potentially used in various applications including bone tissue engineering, cartilage repair and replacement and injectable materials. Fenglan et al. [88] investigated a nano-hydroxyapatite/poly(vinyl alcohol) hydrogel biocomposite which showed good homogeneity and thermal stability and used as a high-performance material for artificial articular cartilage due to the bioactivity the nano-hydroxyapatite provides.
1.3.1.3. Semiconducting nanocomposites

A limited amount of work has been done on semiconducting hydrogel nanocomposites in which quantum dots (QDs) have been incorporated into hydrogel matrices. The strong interest in quantum dots stems from their unique luminescent properties, which leads to potential applications in a wide variety of fields including optoelectronics, biological immunoassays, and biosensors [89]. Pardo-Yissar et al. [90] demonstrated solvent-induced switchable photoelectrochemical functions of hydrogel composites of polyacrylamide and cadmium sulphide that can be used for signal-triggered systems.

1.3.1.4. Magnetic nanocomposites

The incorporation of magnetic nanoparticles such as iron oxide nanoparticles into hydrogels can create tunable nanocomposites which can be remotely controlled by a magnetic field and can be utilized for wide variety of potential applications. These applications range from controlled drug release applications to hyperthermia treatments in cancer patients. For example, Liu et al. [91] developed a gelatin/Fe₃O₄ hydrogel nanocomposite with uniform distribution of magnetic nanoparticles throughout the gelatin matrix.
1.3.1.5. Metallic nanocomposites

It is known that nanometals, especially nanosilver, introduces physical properties such as responsiveness to mechanical, optical, thermal, barrier, sound, magnetic, electric simulation, etc. These unique properties lead to applications in electronics, optics, sensors, actuators, microfluidics sectors, as well as catalysis, separation devices, drug delivery, photonics, biotechnology and medicine.

The general methods used to incorporate nanoparticles into the hydrogels include gelation of preformed nanoparticles in a solution of hydrogel forming monomers [92], embedding of nanoparticles in the hydrogel networks by swelling – shrinking process [93-94], repeated heating, centrifugation, redispersion [95] and entrapment of nanoparticles in hydrogel matrix followed by reduction with common reducing agents. However drawbacks such as aggregation of nanoparticles in monomer solution before and during the gelation process, leaching of nanoparticles out of the network [96], forcing conditions for synthesis and the use of toxic chemicals as reducing agents limit the potential of hydrogel nanocomposites for biomedical applications. These pave the way for biological methods, using plant extracts, which are cost effective and environmental friendly [97]. For example, Siraj Shaik et al. [98] and co-workers reported the insitu formation of AgNPs in starch-co-polyacrylamide hydrogel by using a green
approach in which the Ag$^+$ ions are reduced by *Tridax procumbens* leaf extract. The developed nanocomposites are biodegradable and nontoxic and are potential materials to be used for biomedical applications.

K.S. Kumar et al. [99] proposed a simple and one-step procedure for preparing size-controlled silver nanocomposite hydrogel based on sodium alginate using *Mukia maderaspatna* aqueous leaf extract. H.F. Harrar and co-workers [100] prepared chitosan-g-polyacrylamide hydrogel and spherically shaped AgNPs with average sizes < 26 nm were biosynthesized *insitu* using *Curcuma longa* tuber extract as both reducing and stabilizing agent at room temperature.

Gulsonbi et al. [101] were able to stabilize the silver ions in a semi interpenetrating networks comprised of carboxymethylcellulose – polyacrylamide using *Azadirachta indica*, at ambient temperature. All the prepared hydrogel nanocomposites have shown responsiveness to change in pH of the medium, making it suitable for biomedical applications.

1.3.2. Applications of hydrogel silver nanocomposites

The following are some important fields in which hydrogel silver nanocomposites find applications and a few important regions are given in Fig. 1.3.

- Drug delivery
- Wound dressing
Introduction

- Antimicrobial agent
- Tissue engineering
- Biosensors
- Scaffold
- Biological labelling

Figure 1.3. Applications of hydrogel silver nanocomposites

1.4. Aim of the present investigation

Based on the exhaustive literature survey, the present work is aimed at the development of novel strategy to synthesize hydrogels with certain hydrophilic nature for embedding the silver ions and reducing it to silver
nanoparticles \textit{insitu} using neem plant extract. Therefore, eight different hydrogels of polyacrylamide/polysaccharides, polyacrylamide/protein and polyacrylamide/clay mineral are prepared, their characteristics are studied and applications are investigated.
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